Remarks

Claims 49, 50, 52, 53, 56, 57, 58, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79 and 80-87 are pending. Claims 51, 54 and 55 have been newly cancelled. Claims 49, 50, 52, 53, 56, 57 and 58 have been newly amended. Claims 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79 and 80-87 are newly added. Support for these amendments are found throughout the specification and in the claims as originally filed. No new matter has been entered. All newly added claims are encompassed by Group I of the restriction requirement drawn to methods of identifying biomarkers and methods for diagnosis and prognosis of Chagas disease, further restricted to the CDC14A gene.

Claims 50, 67, 68 and 69 clarify that said levels of RNA encoded by said gene are in blood samples which include all of the types of leukocytes in whole blood, i.e. of blood samples which include granulocytes in addition to mononuclear cells (T-lymphocytes, B-lymphocytes and monocytes). This phrase finds clear support in the specification, including at Figure 5C which shows standardized levels of insulin gene expression in each of the fractions of leukocytes which collectively constitute unfractionated leukocytes, i.e. granulocytes, T-lymphocytes, B-lymphocytes and monocytes (labeled "G.R.", "CD 3+", "CD19" and "MONO", i.e., respectively). It is well known to the ordinarily skilled artisan that CD3 and CD19 are specific cell surface markers of T-lymphocytes and B-lymphocytes (refer, for example, to the enclosed Abstract of Casey *et al.*, 1988. simplified plastic embedding and immunohistologic technique for immunophenotypic analysis of human hematopoietic and lymphoid tissues. Am J Pathol. 131:183-9). The fact that granulocytes (G.R.), lymphocytes [T-lymphocytes (CD 3+) and B-lymphocytes (CD19+)] and monocytes (MONO) represent all of the types of leukocytes found in blood is taught at Fig. A.23 Immunobiology. Garland Publishing. 2001. Fifth Edition. Janeway, Travers, Walport, and Shlomchik, eds. (attached) which clearly teaches that leukocytes are composed of granulocytes and mononuclear cells, and that the latter are composed of lymphocytes and monocytes. Additional support for the term "leukocytes" is found at paragraphs [0004], [0005] and [0088] of the published application (US 2004/0241729).

New independent claim 70 claims a method of classifying gene expression in a test subject relative to a population of control subjects that includes subjects having Chagas disease and healthy subjects. New claim 70 comprises a step of quantifying a level of RNA encoded by an CDC14A gene in a blood sample from the test subject, and a subsequent step of comparing the level in the sample from the test subject with levels of RNA encoded by the gene in blood samples from the subjects having Chagas disease and in blood samples from the healthy subjects. The new claim concludes that a statistically significant determination with a p value less than 0.05 that the level in the sample from the test subject is similar to the levels in the samples from the subjects having Chagas disease and is higher from the levels in the samples from the healthy subjects classifies the level in the sample from the test subject with the levels from the samples from the subjects having Chagas disease; and that a statistically significant determination with a p value less than 0.05 that the level in the sample from the test subject is lower than the levels in the samples from the subjects having Chagas disease and is similar to the levels in the samples from the healthy subjects classifies the level in the sample from the test subject with the levels in the samples from the healthy subjects. Support for reciting comparison of biomarker RNA levels of a test subject with those of control subjects having a disease (i.e. Chagas disease) and with those of healthy control subjects, and determination of a statistically significant difference or similarity therebetween can be found in the published application, for example at paragraph [0127] ("*When comparing two or more samples for differences, results are reported as statistically significant when there is only a small probability that similar results would have been observed if the tested hypothesis (i.e., the genes are not expressed at different levels) were true.*"), and at paragraph [0128] ("*When comparing two or more samples for similarities, results are reported as statistically significant when there is only a small probability that similar results would have been observed if the tested hypothesis (i.e., the genes are not expressed at different levels) were true.*"), respectively. Support for reciting classification of a test subject level relative to specific control levels can be found, for example, at claim 12 as originally filed ("*d) determining whether the level of said one or more gene transcripts of step a) classify with the levels of said transcripts in step b) as compared with the levels of said transcripts in step c)*"), at paragraph [0135] (relating to "*Methods that can be used for class prediction analysis*"), and [0418] ("*Blood samples were taken from patients who were diagnosed symptomatic or*

asymptomatic Chagas disease as defined herein. Gene expression profiles were then analysed and compared to profiles from patients unaffected by any disease.").

Claims Rejection - 35 U.S.C. 112 2nd

Claims 51, 52, 53, 54, 55, 56, and 57 are rejected under 35 U.S.C. 112, 2nd paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention.

The office action indicates that the recitation of "unfractionated samples of lysed blood" is unclear. Although Applicant respectfully traverses, Applicant has canceled independent claim 51 and dependent claims 54 and 55 solely for the purposes of advancing prosecution without prejudice for pursuing the unclaimed subject matter in another application, rendering the rejection of claims 51, 54 and 55 moot. Applicant has amended dependent claims 52, 53, 56 and 57 to be dependent from claim 49 or newly added claim 60, which do not recite the phrase "unfractionated samples of lysed blood".

Claims Rejection - 35 U.S.C. 112 1st

Claims 51, 52, 53, 54, 55, 56, and 57 are rejected under 35 U.S.C. 112, 1st paragraph, as failing to comply with the written description requirement on the grounds that the phrase "unfractionated samples of lysed blood" is new matter. Although Applicant respectfully traverses, Applicant has canceled claim 51 and dependent claims 54 and 55 solely for the purposes of advancing prosecution without prejudice for pursuing the unclaimed subject matter in another application, rendering the rejection of claims 51, 54 and 55 moot. Applicant has amended dependent claims 52, 53, 56 and 57 to be dependent from claim 49 or newly added claim 60, which do not recite the phrase "unfractionated samples of lysed blood".

Claims 49, 50, 51, 52, 53, 54, 55, 56, and 57 are rejected under 35 U.S.C. 112, 1st paragraph, as failing to comply with the enablement requirement.

Applicant respectfully traverses. Applicant disagrees with the rejection's assertion that the skilled artisan would have required an undue amount of experimentation to make and/or use

the claimed invention in view of the breadth of the claims, the amount of guidance provided by the specification, and the level of predictability in the art.

The rejected claims include the steps of determining the level of RNA encoded by an CDC14A gene in a blood sample obtained from a human test subject and comparing it to the level of control RNA encoded by the gene in blood samples of control subjects, where the comparison is indicative of Chagas disease in said human test subject.

Applicant specifically traverses the statement on page 4 of the office action that "*the independent claim, as written, states that a comparison of a human test subject CDC14A RNA level in a blood sample to a control indicates that Chagas disease is present in the test subject*", and the statement on page 5 of the office action '*The claims are extremely broad because they... set forth that any or all comparison between a test subject and RNA level from "control subjects" is indicative of disease*'. Applicant clarifies that the phrase "wherein said comparison of said quantified level of step (a) with said quantified level of said control subjects is indicative of Chagas disease in said human test subject" of independent claim 49, is a narrowing limitation, limiting the claim to only those comparisons which are indicative of the test subject having Chagas disease, and excluding those comparisons which do not indicate that the test individual has Chagas disease.

However, in the interest of expediting prosecution, Applicant has added new claims which more clearly set forth the subject matter of the newly cancelled claims. Specific points raised in the instant enablement rejection will be addressed to the extent that they are relevant to the newly added claims.

The rejection asserts that the claims are broad with respect to "control subjects", indicating that "control subjects" could encompass patients with Chagas disease, healthy patients, patients with some other disease such as depression, rheumatoid arthritis or multiple sclerosis (page 5 of the office action). The instant claims recite two clearly defined sets of controls; subjects having Chagas disease and healthy controls. At least one claim, claim 63, limits the controls to healthy subjects.

The office action states that the claims do not "set forth the direction of the difference necessary to indicate Chagas disease" (page 5 of the Office Action) and suggests that without

providing this information, the mere observation of differences is an unpredictable indicator of Chagas disease.

The Applicant respectfully submits that the invention is taught in such terms that one skilled in the art can make and use the claimed invention, including the use of the elected biomarker CDC14A as an indicator of Chagas disease as described in the claims without disclosing the direction or the level of difference that exists between patients having Chagas disease and individuals not having Chagas disease. The Applicant has identified the elected gene CDC14A as being more highly expressed in individuals diagnosed as having Chagas disease relative to healthy controls by demonstrating a statistically higher level of RNA. The statistical significance of the differential expression of CDC14A is evidenced by its P value of 0.0492, as listed in Example 28 (Table 3Z). Therefore the Applicant has taught that there is a significant difference in differential expression for CDC14A as between a population of individuals having Chagas disease and a population of individuals not having Chagas disease, and further has taught to compare the level of expression of CDC14A in a test individual with populations having Chagas disease and populations not having Chagas disease using classification methods to determine the similarity or difference in gene expression levels as between the test subject and the tested populations (see paragraphs [0127] to [0128] and [0131] to [0136] in addition to [0422]. Furthermore, the Applicant contends that it does not require undue experimentation for one of skill to determine the inherent direction or level of the statistically significant differential expression required for the claimed methods of detecting a Chagas disease, given the widely established and validated analytical tools for analyzing gene expression levels. Therefore, it is not necessary for the Applicant to have taught the exact direction or level of difference between the two populations for one of skill to practice the invention. The Applicant has provided sufficient information by teaching that there CDC14A is differentially expressed and that the differential expression between healthy and control subjects is significant as between the populations.

Nevertheless, in the interest of expediting prosecution of the instant application, Applicant currently elects to amend claim 49 and to file new claims 64, 65, 66, 70 and 76 which limit the differential expression to a higher expression in disease subjects with a fold-change of

at least 1.5, and to file new claim 80 limiting the fold-change to 2 or less, in accordance with the data presented in Example 28 and Table 3Z of the specification.

The Office Action also contends that the claims are not enabled due to unpredictability in the art on the grounds that Tsuang *et al.* teaches experiments which are similar to those of the specification relating to Chagas disease, and teaches that such experiments must be interpreted with caution due to various potential limitations. Applicant respectfully submits, however, that the preponderance of the teachings of Tsuang *et al.* are nevertheless clearly in favor of the experimental data disclosed therein being reliable. In particular, Tsuang *et al.* clearly teaches that the results are most likely reliable despite the limitations cited by the Examiner, in accordance with the citation: "*Despite these limitations, this work demonstrates the potential utility of blood-based RNA profiling as a diagnostic tool...*" (concluding paragraph of Tsuang *et al.*). Applicant further submits that the experimental results disclosed in Tsuang *et al.* should enjoy a strong presumption of validity in view of this reference being a high-level and peer-reviewed academic publication. Applicant wishes to point out that the cautionary statements set forth in Tsuang *et al.* which were cited by the Examiner clearly represent a maximally conservative interpretation of the data, in line with the maximally conservative standards, for example, of the U.S. FDA for approval of novel medical applications to humans. The Applicant respectfully indicates that it is improper to incorporate the standards for use by the FDA for purposes of determining patentability (see for example Application of Anthony, 56 C.C.P.A. 1443, 414 F.2d 1383, 162 U.S.P.Q. (BNA) 594 (1969); "*We believe that Congress has recognized this problem and has clearly expressed its intent to give statutory authority and responsibility in this area to Federal agencies different than that given to the Patent Office. This is so because the standards established by statute for the advertisement, use, sale or distribution of drugs are quite different than the requirements under the Patent Act for the issuance of a patent.*"

The office action specifically contends that CDC14A expression in blood may not be indicative of Chagas disease on the grounds that Showe *et al.* teach that CDC14A is differentially expressed in blood samples from cutaneous T-cell lymphoma patients relative to healthy controls.

Applicant respectfully disagrees that the teachings of Showe *et al.* are relevant to the claims, and submits that the cited teachings of Showe *et al.* are in fact drawn to subject matter which is not encompassed by the claims. Namely, the data set forth by Showe *et al.* only relates to genes which are differently expressed in peripheral blood mononuclear cells (PBMCs or "mononuclear cells" ; i.e. lymphocytes and monocytes), hence in leukocytes which are fractionated into cell types. This is clearly evidenced, for example, in the materials and methods section of Showe *et al.* in the title, subtitle and first paragraph of Example 1 describing RNA sample isolation, in accordance with the recitations: "*Analyses of PBMC From Patients With CTCL*" (title of Example 1), "*Purification of PBMC from CTCL Samples and Preparation of Normal Controls*" (subtitle of Example 1), and "*PBMC were obtained by Ficoll gradient separation from peripheral blood of both normal volunteers and leukemic phase CTCL...*" (paragraph [0142]). It is well understood in the art that such Ficoll fractionation is employed to isolate PBMCs from granulocytes (refer, for example, to enclosed Fig. A.23. Immunobiology. Garland Publishing. 2001. Fifth Edition. Janeway, Travers, Walport, and Shlomchik, eds.), granulocytes being the majority cell component of leukocytes (refer, for example, to enclosed Complete Blood Count (CBC) table. Merck Manual Home Edition, 2006). In relevant contrast, the claims are drawn to RNA of samples which comprise unfractionated leukocytes, i.e. which comprise granulocytes as well as the minority mononuclear cell fraction of leukocytes. Thus, in setting forth data relating only to genes which are differentially expressed in a minority fraction of leukocytes, Showe *et al.* fails to provide teachings relating to genes which are globally differentially expressed in unfractionated leukocytes, as required by the claims. Namely, unfractionated leukocytes further comprise a majority fraction of granulocytes which are a distinct cell type relative to mononuclear cells and which inherently have distinct gene expression profiles relative to mononuclear cells [refer, for example to enclosed abstract of Hashimoto S. et al., 2003. Gene expression profile in human leukocytes. Blood 101:3509-13; and to Figure 5C of the instant specification which indicates significantly different expression levels of an exemplary gene between granulocytes ("G.R.") and mononuclear cells, the latter being represented by cumulative levels of the combination of B-lymphocytes ("CD19"), T-lymphocytes ("CD3") and monocytes ("MONO")]. Thus, it cannot be predictably extrapolated that the global differential expression of any given gene (such as CDCA1 between healthy

control subjects and SLE patients) observed in Ficoll-fractionated mononuclear cells, as taught by Showe *et al.*, will also be observed in unfractionated blood cells, as required by the claims.

Thus Showe *et al.* fails to teach that there is a level of unpredictability in the art such that the claims are not enabled. Nevertheless in order to more clearly set forth the differences between the claimed subject matter and the prior art, a described above, instant claims 50, 67, 68 and 69 clarify that said levels of RNA encoded by said gene are in blood samples which include all of the types of leukocytes in whole blood.

In addition, solely for the purpose of expediting prosecution, Applicant has included the limitation in claims 64 and 66 that the recited comparison between a test subject and controls indicates that the test subject is a "candidate" for having Chagas disease. Applicant has also added new claim 70 which is a method of classifying CDC14A gene expression in a test subject with that in Chagas disease patients or healthy control subjects, and has added new claim 76 which is a method of identifying a gene encoding a CDC14A gene as a candidate biomarker for Chagas disease in a human subject.

The rejection contends that the claims may not be enabled due to insufficient replication of experimental results, on the grounds that that Lee teaches that data obtained from microarrays must be replicated in order to screen out false positive results (page 7 of the Office Action), on the grounds that post-filing reference Tsuang *et al.* teaches that the results of experiments such as those disclosed in the specification relating to differential expression of CDC14A in blood of Chagas disease patients may be intrinsic to the cohort studied (page 8), and on the grounds that Newton *et al.* teaches that "replication of data is critical to validation" (page 11).

Applicant respectfully disagrees that insufficient replication is provided by the specification. Nevertheless, in the interest of expediting prosecution of the instant application, Applicant currently elects file the attached declaration under 1.132 which discloses post-filing validation experiments using quantitative RT-PCR (QRT-PCR), an alternate technology relative to microarray analysis employed in the experiments disclosed at Example 28 and Table 3Z of the specification, as well as using an independent cohort of control and disease subjects relative to those employed in the experiments disclosed at Example 28 and Table 3Z of the specification. The experiments disclosed in the declaration clearly show that RNA encoded by the gene

CDC14A is present at 1.6-fold higher levels in blood of subjects having Chagas disease relative to healthy control subjects with a statistical significance of p equal to 0.033.

The office action states on page 7 that Wu et al (2001) teaches that gene expression data, such as microarray data, must be interpreted in the context of other biological knowledge, and that the conclusions that can be drawn from a given set of data depend on the particular choice of data analysis.

Applicant respectfully disagrees and submits that gene expression which is reproducible, and is correlated with the state of health or disease of the individual does not necessarily result directly from the state of disease of the individual. Rather these changes in expression may simply represent a downstream side-effect of pathogenic processes, and it is not necessary that the biological relevance of the data be known to allow this difference in expression to be useful as a biomarker. For example prostate-specific phosphatase and prostate-specific antigen (PSA) were long used as biomarkers without an understanding of their function (refer, for example, to the enclosed abstracts of: Chu TM, 1990, Prostate cancer-associated markers. Immunol. Ser. 53:339-56; and Diamandis EP., 2000, Prostate-specific antigen: a cancer fighter and a valuable messenger? Clin Chem. 46:896-900).

The Examiner also argues, on the basis of post-filing art of Wu (2001) and Newton (2001), that many factors may influence the outcome of the data analysis and notes that conclusions depend on the methods of data analysis. While considerations such as variability, and normalization are of importance, these considerations are well understood by a person skilled in the art and have been applied for many years to permit development of biomarkers which are indicative of disease. These challenges are well understood, as are the routine experiments required to exemplify statistically significant differences in populations.

As grounds for lack of enablement due to a high level of unpredictability, the office action states on page 10 that Cheung et al. teaches that the natural variation in gene expression amongst different individuals is such that it is unpredictable as to whether or not any level of altered CDC14A gene expression will be indicative of a presence or absence of Chagas disease.

Applicant respectfully disagrees that Cheung *et al.* constitutes grounds for non-enablement of the claims Firstly, the results disclosed by Cheung *et al.* cannot be reliably

extrapolated to primary blood samples since the lymphoblastoid cells employed by Cheung *et al.* are significantly modified relative to primary blood cells, due to being cultured cell lines generated by immortalization of primary human cells derived from "CEPH" families, as indicated in Reference no. 10 of Cheung *et al.* (Dausset *et al.*, 1990. Genomics 6:575; enclosed) at p. 575, right column, 1st paragraph. Applicant notes that immortalized cultured cell lines such as the lymphoblastoid cells taught by Cheung *et al.* undergo significant genetic modification such as strong genome-wide demethylation (refer, for example, to enclosed abstract of: Vilain *et al.*, 2000. DNA methylation and chromosome instability in lymphoblastoid cell lines. Cytogenet Cell Genet. 90:93), as a result of extensive *in-vitro* culturing in the absence of immune or apoptotic mechanisms which function to eliminate mutated cells in the body. As such, immortalized CEPH lymphoblastoid cells may represent a particularly unsuitable cell type for modeling gene expression variability in primary blood cells. To the extent that Cheung et al. could still be considered to suggest that larger populations of diseased and control populations may be useful to determine what level of differential expression is indicative of disease amongst the population at large, Applicant submits that the extension of the experiments as outlined in the specification to additional individuals is merely routine. As is noted in Re Wands "*even a considerable amount of experimentation is permissible to practice the claimed methods, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed.*" (Re Wands, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988)).

Furthermore, the decision *In re Angstadt*, 190 U.S.P.Q. 218 (C.C.P.A. 1976) clearly states that even in an unpredictable art, and clearly permits the presence of a screening step to identify those embodiments which possess the desired activity is permissible. In fact, in *Angstadt*, the Court specifically dismissed the notion that the specification must provide a level of guidance that would predict the outcome of an experiment "with reasonable certainty before performing the reaction" and that "such a proposition is contrary to the basic policy of the Patent Act, which is to encourage disclosure of inventions and thereby to promote progress in the useful arts." The "predictability or lack thereof" in the art refers to the ability of one skilled in the art to extrapolate the disclosed or known results to the claimed invention.

Applicant wishes to point out that in *In re Wands*, the court stated that "[e]nablement is

not precluded by the necessity for some experimentation such as routine screening. However, experimentation needed to practice the invention must not be undue experimentation. 'The key word is 'undue' not 'experimentation' (citing *In re Angstadt*, 537 F. 2d 498 at 504, 190 U.S.P.Q. 214 at 219 (C.C.P.A. 1976)). The Court also stated that "the test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed." (citing In re Jackson, 217 U.S.P.Q. 804 at 807 (Bd. App. 1982)).

As such Applicant believes there is sufficient guidance provided by the specification and that the art is sufficiently predictable such that the amount of experimentation to perform the subject matter within the instant claims is not undue.

In light of the amendments and above remarks, Applicant contends that the claims are fully enabled, and respectfully requests reconsideration and withdrawal of the instant rejections.

Conclusion

Applicant submits that all claims are allowable as written and respectfully request early favorable action by the Examiner. No new matter is added. If the Examiner believes that a telephone conversation with Applicant's attorney/agent would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney/agent of record.

Respectfully submitted,

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Abstract of Casey et al., 1988. simplified plastic embedding and immunohistologic technique for immunophenotypic analysis of human hematopoietic and lymphoid tissues. Am J Pathol. 131:183-9;

Abstract of: Chu TM, 1990, Prostate cancer-associated markers. Immunol. Ser. 53:339-56;

Abstract of: Diamandis EP., 2000, Prostate-specific antigen: a cancer fighter and a valuable messenger? Clin Chem. 46:896-900);

Abstract of: Hashimoto S. et al., 2003. Gene expression profile in human leukocytes. Blood 101:3509-13

Abstract of: Vilain et al., 2000. DNA methylation and chromosome instability in lymphoblastoid cell lines. Cytogenet Cell Genet. 90:93;

Complete Blood Count (CBC) table. Merck Manual Home Edition, 2006;

Article of Dausset et al., 1990. Genomics 6:575; and

Fig. A.23. Immunobiology. Garland Publishing. 2001. Fifth Edition. Janeway, Travers, Walport, and Shlomchik, eds.